STUDIES ON SHELL FORMATION. II. A MANTLE-SHELL PREPARATION FOR IN VITRO STUDIES[1]

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This report describes a preparation, consisting of an oyster mantle and its associated shell, which has been developed for studying mechanisms of shell formation *in vitro*. The preparation has been found capable of depositing shell of normal structure and can be easily maintained for periods of several days. An account of shell formation by the mantle-shell preparation is presented.

Methods

All experiments were carried out with the oyster *Crassostrea virginica* Gmelin.[3] The mantle-shell preparation is isolated in the following manner. The oyster is placed on its left valve and the hinge is gently pried open with an oyster knife or scalpel. The knife is then twisted in the hinge in order to open the valves slightly, and the adductor muscle is severed in the middle with a razor blade. The opening of the valves should be kept at a minimum during this operation so that the mantle is disturbed as little as possible. Unlike many of the bivalves, the mantles of the oyster are fused along the base of the gills. The right valve is lifted slightly, and beginning at the posterior end and working toward the hinge, the right mantle is snipped free from the left mantle, the gills, and the visceral mass with small scissors. The organs attached to the left mantle can next be removed. One now has the two valves with their attached mantles completely intact. When the dissection is performed with sufficient care both mantles remain attached to the shell and retract only slightly. On being placed in sea water the mantles relax and cover the entire shell surface (Fig. 1).

The mantle-shell preparations were suspended in running sea water by means of a plastic V-shaped clamp with the mantle downward. In this position mucus is eliminated more efficiently.

To establish the ability of the mantle to deposit shell *in vitro*, two or three fragments of glass coverslips were inserted between the mantle and the shell following the method of Brooks (1905) for the whole oyster. This can be conveniently performed by lifting a small portion of the mantle edge with a small spatula and inserting a coverslip fragment. Even in the inverted position, the coverslip fragments were firmly pressed against the shell surface by the mantle. The coverslip fragments were removed after the desired interval, thoroughly washed in distilled water to remove dissolved salts, and examined under the light field and polarizing microscopes.

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[3] Previously *Ostrea virginica*. Re-named by Gunter (1950).

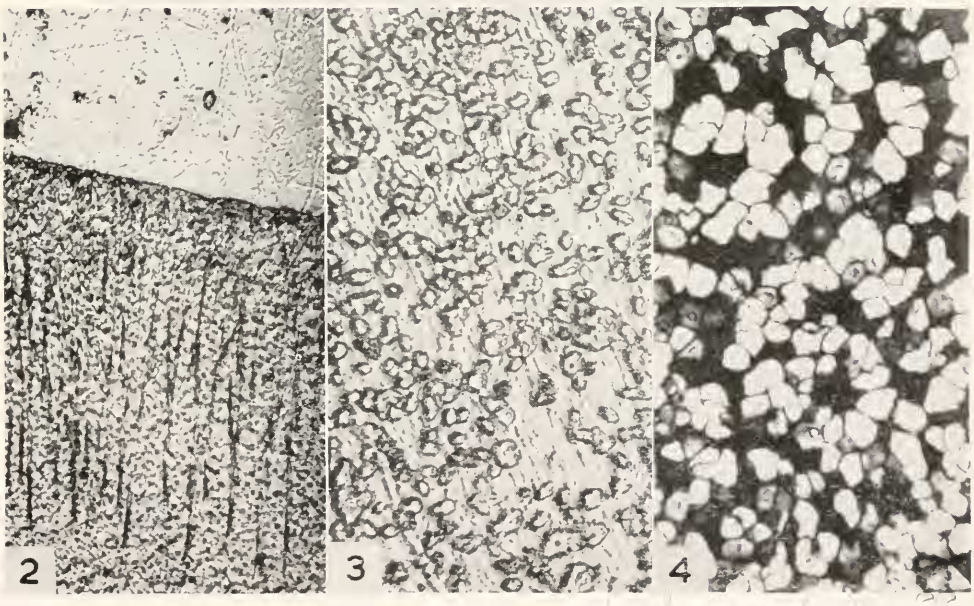

FIGURE 1. Mantle-shell preparation of a right shell.

FIGURE 2. Area below the diagonal line represents deposits by the mantle-shell preparation on a coverslip fragment over a period of 62 hours. The coverslip was inserted between the mantle and the shell 74 hours after its isolation. The marginal fibrous zone is clearly distinguishable, but the other two zones cannot be easily distinguished without the aid of the polarizing microscope.

FIGURE 3. Crystalline deposits of the innermost zone (structureless). Lower zone of Figure 2 magnified.

FIGURE 4. The zone of honeycomb pattern photographed between crossed Nicol prisms. Intermediate zone of Figure 2 magnified.

These experiments were performed during June and July, 1950. The temperature of the sea water was 26–30 degrees C. and the salinity range was 31 to 36 parts per thousand.

Results

The mantle-shell preparations remained in good condition for as long as seven days, as indicated by normal ciliary movement and retraction of the mantle edge on mechanical stimulation. More recently, Jodrey (1953) has found the respiration to be normal for the same duration. Longer periods of survival were not investigated.

The examination of pieces of coverslip inserted between the mantle and shell after various intervals revealed that a sheet of organic material was first elaborated followed by the deposition of birefringent crystals. These crystals appeared eight hours after insertion and produced bubbles in dilute acid. These increased in size as well as in number and became contiguous (Fig. 3). Within about 2½ days an organized shell structure began to appear. After three days the deposits showed three main concentric zones of organic material (Fig. 2): (1) a radiating fibrous zone at the outer margin; (2) a zone of honeycomb pattern central to this; and (3) a structureless sheet innermost. The fibrous zone consisted of an organic sheet with radiating fibers running parallel to the surface and perpendicular to the periphery of the mantle. The honeycomb zone showed cellular-like structure probably with open upper surfaces since some of the small areas contained crystals whereas others did not. Except for the absence of fibers the innermost zone resembled the fibrous zone. The fibrous and honeycomb structures were much thicker than the structureless organic sheet and together measured about one mm. in width where oysters of 10 cm. in length were used. The thickness of the organic deposits gradually decreased toward the center of the shell. The three zones resembled the structure of the periostracum, prismatic and nacreous layers of normal shell except that the polyhedral units of the periostracum were lacking.

Birefringent crystalline materials were deposited on all three zones, the most dense being on the honeycomb area. As a result, this region is the most distinct of the three regions when observed between crossed Nicol prisms (Fig. 4). Crystals were imbedded in the cellular framework of the honeycomb region, but were simply scattered on the other two zones. With treatment in 0.001 N HCl the crystalline material dissolved while the organic structures remained intact.

Not all of the preparations were capable of depositing crystals within the 8-hour period mentioned, but pieces of coverslip left in place for more than 40 hours were rarely without crystalline deposits. By introducing the glass fragments at intervals it was found that crystal deposition was possible even after four days of isolation. However, the time required for deposition was longer than with a recently isolated mantle. While birefringent crystals were deposited by all areas of the mantle, the rate of deposition near the muscle was slower than near the margin. This was confirmed later by the use of Ca^{45} (Jodrey, 1953).

Discussion

Studies of shell formation *in vivo* by Bevelander and Benzer (1948), confirming the observations of earlier workers, showed that the organic matrix is first de-

posited as a thin sheet and comes to be arranged in prisms and striae in later development. Granules from the mucus cells are deposited in the organic matrix and undergo crystal growth and continue to grow eventually into arrangements whereby the crystals come to be enclosed in a thin layer of organic matrix and assume a polyhedral shape (in *Pinna*).

Bevelander and Martin (1949) reported that isolated strips of mantle of *Pintata radiata* can be maintained *in vitro* and will produce crystals in the course of several days. In the mantle-shell preparation described here the association of mantle and shell of the intact oyster is maintained; and both organic and crystalline shell components are elaborated in patterns closely resembling the normal. This synthetic capacity, together with the stability *in vitro,* emphasizes the utility of the preparation for the study of shell-forming mechanisms of the mantle. The potentialities are still further increased by the use of radioisotopes for quantitative measurements of shell formation (Wilbur and Jodrey, 1952).

The mantle-shell preparation forms shell more slowly than the whole oyster as shown by crystal formation on coverslip fragments inserted beneath the mantle and by the rate of calcium deposition as measured by Ca^{45} (Jodrey, 1953). Other differences may also become evident as shell formation *in vitro* is studied in more detail. Differences between the mantle-shell preparation and the intact oyster must be considered a fortunate circumstance, for by their study and correction through experimental procedures, information concerning factors operative in the formation of shell should result.

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Summary

1. A preparation consisting of an oyster mantle and its attached shell has been devised for *in vitro* studies of shell formation. Shell consisting of typical organic matrix and birefringent crystalline material was elaborated by this preparation, though at a slower rate than in the intact oyster.

2. All parts of the mantle were shown to be capable of shell formation *in vitro.*

3. The isolated mantle-shell preparation can be maintained in good condition for several days.

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